

Applicant(s): Lowe, et al.
Application No.: 09/463,801
Filing Date: July 28, 2000
Docket No.: 1374-4 PCT/US
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AMENDMENTS TO THE SPECIFICATION:

On Page 1, line 1, please change the title of the application to the following amended title:

Medium Means for Producing and/or Treating Alcoholic Beverages, Especially Wine or Sparkling Wine, as Well as Its Applications

Please replace the paragraph starting on page 1, line 5 of the filed application with the following amended paragraph:

Description **BACKGROUND OF THE INVENTION**

Field of the Invention

The invention refers to means for producing and/or treating alcoholic beverages, especially wine or sparkling wine, in accordance with the preamble of the main claim as well as its applications.

Please replace the paragraph starting on page 1, line 10 of the filed application with the following new paragraph:

Description of the Prior Art

Various species of microorganisms, especially yeast used for alcohol fermentation, are employed in the production of alcoholic beverages, especially of wine or sparkling wine. To optimize the results, other species of microorganisms and enzymes are added to the product or its preliminary stages. Thus, for example, lactic acid bacteria serve to break down malic acid and pectinases accelerate must clarification.

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Please replace the paragraph starting on page 2, line 11 of the filed application with the following amended paragraph:

US 4,996,150 ~~4,996,150~~ describes a process for the microencapsulation of biocatalysts, preferably yeast, as well as their use in the continuous production of ethanol. The biocatalysts are contained in a matrix of an anionic polysaccharide and a cationic polymer. Here, too, the microcapsules do not have any cell-free covering membrane, so that yeast out-growth cannot be sufficiently prevented. Additionally, it is hardly possible to immobilize the smaller biocatalyzers, such as enzymes, with sufficient surety.

Please replace the paragraph starting on page 3, line 9 of the filed application with the following new paragraph:

OBJECTS AND SUMMARY OF THE INVENTION

The object of the invention is to make available means for producing and/or treating alcoholic beverages, especially wine or sparkling wine, according to the preamble of the main claim, in which the cells and/or enzymes are permanently immobilized, in which the permeability of the mechanical stability of the covering membrane is adjustable, and in which the contents of the microcapsules can be liquefied.

Please replace the paragraph starting on page 3, line 17 of the filed application with the following new paragraph:

~~The object will be evidenced by means with the characteristics of claim 1 as well as by the applications according to claims 20 and 21, whereby the sub-claims address the advantageous aspects of the invention.~~

In accordance with one form of the present invention, a microcapsule for treating a first reactant with one or more further reactants selected from the group consisting of

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microorganisms and enzymes to yield one or more products includes a core including the one or more further reactants, and a membrane including a first layer that fully encloses the core and a second layer that fully encloses the first layer. The membrane is configured and adapted to be impermeable to the one or more further reactants and is adapted to be permeable to the first reactant and to at least one of the one or more products.

In accordance with another form of the present invention, a method for treating a first reactant with one or more further reactants selected from the group consisting of microorganisms and enzymes to yield one or more products includes at least the step of immersing one or more microcapsules in the first reactant. At least one of the microcapsules includes a core having the one or more further reactants, and a membrane including a first layer that fully encloses the core and a second layer that fully encloses the first layer. The membrane is configured and adapted to be impermeable to the one or more further reactants and is adapted to be permeable to the first reactant and to at least one of the one or more products.

These and other objects, features and advantages of the present invention will be apparent from the following detailed description of illustrative embodiments thereof, which is to be read in connection with the accompanying drawings.

Please replace the paragraph starting on page 3, line 20 of the filed application with the following new paragraph:

BRIEF DESCRIPTION OF THE DRAWING

Figure 1 is a simplified schematic diagram of a continuous apparatus for alcohol production constructed in accordance with one form of the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

In accordance with the present invention, the The species of microorganisms and/or

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enzymes used in the production and/or treating of alcohol beverages, especially wine or sparkling wine, are immobilized in that they are contained in the interior of the capsule, and that a covering membrane completely surrounds the interior of the capsule. The covering membrane for these microorganisms and/or enzymes is not permeable, thus preventing the escape of the microorganisms or enzymes. In order to ensure a substance conversion, the covering membrane for the substances to be converted (eductes) is permeable, also included in this are the nutrients necessary for the microorganisms, for example, glucose; the covering membrane cartridge is also permeable to at least a part of the produced or converted substances (products), alcohol and carbon dioxide, for example. The requirements of permeability and mechanical stability are fulfilled by the covering membrane that has at least two layers, radially arranged on top of each other, whereby each layer completely encloses all of the radially arranged layers beneath it. Advantageously the individual layers are bound together ionically and/or covalently.

Please replace the paragraph starting on page 4, line 21 of the filed application with the following amended paragraph:

According to an advantageous embodiment, the cells and/or enzymes contained in the inner part of the microcapsules are embedded in a matrix. This matrix can be constructed from an alginate compound with a polyvalent cation, for example, calcium, strontium, barium, aluminum and/or iron.

Please replace the paragraph starting on page 12, line 11 of the filed application with the following amended paragraph:

Further details of the apparatus can be found in the following descriptive section in which, with the drawing, an example of an embodiment is described in more detail. The attached figure (Figure 1) shows the schematic structure of a continuous apparatus for alcohol

production. The liquid to be fermented is taken from a storage tank (1) and put into a mixer (2), where it mixes together with a liquid backflowing from the still (4). This mixture is converted into alcohol in the bioreactor (3) from the yeast immobilized in the medium according to the invention. The alcohol-containing fluid is put into a still (4). From there, an alcohol-containing gas phase is routed into a distillation column (5). There, the different boiling points of alcohol in contrast to water are used to enrich the alcohol that is gathered in a collection tank for the primary product (6). The alcohol-lacking portion of the pre-fermented liquid is taken out of the still and cooled in a heat exchanger (8) and routed back into the mixer (2). In order for the liquid in circulation to contain a minimum level of the substances to be converted, especially glucose, a part of the watery portion is regularly removed from the still (4) and gathered as a by-product in a collection tank (7).

Please replace the paragraph starting on page 12, line 29 of the filed application with the following amended paragraph:

5 g sodium alginate (Kelco Co., Hamburg) are dissolved in 700 ml water. Then 70 g of dry yeast was subsequently stirred into this solution (Oenoform, Erbslöh Co., Geisenheim). This suspension was dropped into a 0.6% solution of calcium chloride. After a few minutes of curring time, the beads containing the yeast cells in a calcium-alginate matrix were first washed with water and subsequently with an aqueous 0.05% solution of polyethylenimine (mean molecular mass 1 mil, Fluka Co.) and was afterwards washed with an aqueous 0.06% solution of carboxymethyl cellulose (mean viscosity, Fluka Co.). Subsequently, the microcapsules thus obtained were washed with water and once again put into the polyethylenimine and carboxymethyl cellulose solution. After being cleaned with water, the microcapsules were stored in water. The microcapsules have a two-layered membrane cartridge, whereby each layer consists of the polyelectrolyte complex of polyethylenimine and carboxymethyl cellulose. Because the calcium alginate beads comprising the cells were produced first and because the layers of covering membrane were subsequently applied, the covering membrane layers showed no yeast cells that could grow out of the microcapsules.

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The yeast thus immobilized showed the same activity as yeast immobilized in unlayered calcium-alginate beads.

Although illustrative embodiments of the present invention have been described herein with reference to the accompanying drawing, it is to be understood that the invention is not limited to those precise embodiments, and that various other changes and modifications may be effected therein by one skilled in the art without departing from the scope or spirit of the invention.

On Page 19, line 1, please delete the title as shown below:

~~MEDIUM FOR PRODUCING AND/OR TREATING ALCOHOLIC BEVERAGES,
ESPECIALLY WINE OR SPARKLING WINE, AS WELL AS ITS APPLICATIONS~~

A substitute specification, incorporating the amendments stated above, is submitted herewith for the Examiner's convenience. A marked-up copy of the specification showing the changes which were made is also submitted herewith.